17β-Estradiol attenuates intimal hyperplasia and macrophage accumulation with a reduction in monocyte chemoattractant protein 1 expression in a vein graft model

Masaaki Ryomoto, MD,[a] Randal A. Wolff, PhD,[b] Jeffrey J. Tomas, BS,[b] Takashi Miyamoto, MD,[a] and **John R. Hoch, MD,[b]** *Madison, Wis*

Objective: **Autogenous vein grafts are commonly used for arterial reconstructive procedures. Their success is limited by the development of intimal hyperplasia, a fibroproliferative disease that predisposes the grafts to occlusive stenosis. Our goal was to assess whether 17β-estradiol (E_2) inhibits vein graft intimal hyperplasia coincident with a reduction in monocyte chemoattractant protein 1 (MCP-1) expression and macrophage accumulation.**

Method: **Male Lewis rats were implanted with time-release pellets that contained 0.5 mg E_2 (E5 group) or placebo (PL group). Epigastric vein to common femoral artery interposition grafts were harvested at 2, 4, 8, and 12 weeks after surgery. We assessed macrophage/monocytes numbers, proliferating cell nuclear antigen, MCP-1, and transforming growth factor-β1 with use of immunohistochemistry. MCP-1 message expression was quantified by real-time polymerase chain reaction.**

Results: **The time-release pellets raised the serum E_2 level to greater than 250 pg/mL on the day of surgery. Serum E_2 level declined to 43 ± 13 pg/mL by 4 weeks and to baseline by 6 weeks. We found that the neointimal area ratio was reduced significantly in the E5 group at 2 and 4 weeks (45%, $P < .05$, and 68%, $P < 0.05$, respectively) relative to that in the PL group. The number of proliferating cells was reduced in the E5 group. There was a significant attenuation of MCP-1 expression and of the number of macrophages accumulating in the graft with E_2 treatment. Furthermore, MCP-1 messenger ribonucleic acid expression was also significantly attenuated in the E5 group at 4 weeks when compared to the PL group. There was no significant difference between the two groups in the expression of transforming growth factor-β1.**

Conclusions: **E_2 treatment reduces vein-graft intimal hyperplasia coincident with a reduction in MCP-1 expression, macrophage accumulation, and cell proliferation. (J Vasc Surg 2002;36:613-21.)**

It is a well-established procedure to use autogenous vein for peripheral arterial reconstruction rather than prosthetic conduits because of the resulting higher patency rate.[1] However, during the first 5 years, the primary autogenous vein-graft patency rate for infrainguinal arterial reconstruction has been reported as 62% to 72%.[1-4] Vein graft patency is limited by the development of intimal hyperplasia (IH). Graft stenoses commonly occur at the perianastomotic region, where additional injury to the endothelial cell lining by the anastomotic procedure may be an important stimulus to an abnormal wound healing response. Central to wound healing is the macrophage, whose cytokines can influence monocyte chemotaxis, induction of smooth muscle cell proliferation and migration, and extracellular matrix production. Endothelial cell and vascular smooth muscle cell (VSMC) injury may stimulate the release of monocyte chemoattractant protein 1 (MCP-1).[5] MCP-1 is one of the pivotal chemokines in the vessel wall, and release of this chemokine causes macrophages to migrate and accumulate at anastomotic sites. We previously described MCP-1 expression and subsequent macrophage accumulation coincident with the development of rat vein graft IH.[6]

Epidemiologic data suggest that the incidence of cardiovascular diseases is lower in premenopausal women or postmenopausal women receiving estrogen replacement therapy, than in men or postmenopausal women not taking supplemental estrogen.[7] In a clinical setting, it has been reported that pretreatment with 17β-estradiol (E_2) can limit leukocyte activation in men after elective coronary artery bypass graft surgery.[8] The mechanism of estrogen's cardioprotective effects is unknown. Pervin et al[9] reported that treatment with E_2 resulted in a reduction in aortic atherosclerosis in rats fed a high cholesterol diet. The investigators attributed the reduction in atherosclerosis to reduced expression of MCP-1.[9] Although E_2 treatment can reduce atherosclerosis and IH in an aortic allograft model[10]

From the Department of Thoracic and Cardiovascular Surgery, Hyogo College of Medicine,[a] and the Division of Vascular Surgery, Department of Surgery, University of Wisconsin-Madison, Medical School.[b]

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Reprint requests: John R. Hoch, MD, Department of Surgery, Clinical Science Center G5/325, 600 Highland Ave, Madison, WI 53792.

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and in arterial balloon injury models,[11-13] no successful results have been reported in an in vivo vein graft model.

In the present study, we demonstrate that estradiol treatment can reduce vein graft IH coincident with a reduction in MCP-1 expression and macrophage migration and accumulation.

METHODS

Treatment, vein grafting, and harvest. The statistical experimental design was completely randomized. Subject animals received randomized treatment and time assignments by using computer-generated random numbers. This method ensures that biases between experimental groups will, on average, be zero. Two days before surgery, male Lewis rats (weight, 350-450 g) were implanted subcutaneously with time-release pellets (Innovative Research of America, Sarasota, Fla) containing 0.5 mg of E_2 (E5 group) or placebo (PL group). Epigastric vein to common femoral artery interposition grafts were placed via aseptic microsurgical techniques as described previously.[14] In brief, each animal was anesthetized intraperitoneally with ketamine and xylazine, and the epigastric vein was dissected from the surrounding tissue. An 8-mm segment of relaxed epigastric vein was excised, reversed, and interposed into a 3-mm arterial defect. Each anastomosis was completed with 8 to 10 interrupted sutures of 10-0 nylon. After a 30-minute total ischemic time, hemostasis was restored, patency was confirmed, and the wound was irrigated and closed. The animal was observed closely until recovery. Provision of animal care complied with the *Principles of Laboratory Animal Care* (formulated by the National Society for Medical Research) and the *Guide for the Care and Use of Laboratory Animals.* Procedures were approved by the Research Animal Resources Center at the University of Wisconsin-Madison.

Harvest time points in this study were 2, 4, 8, and 12 weeks. For quantitative analysis of neointimal area ratio, hematoxylin and eosin stains of 5 to 10 grafts from each treatment (E5 or PL) at each time point were examined. Sections from these grafts were also used for immunohistochemical analysis of ED1, proliferating cell nuclear antigen (PCNA), MCP-1, and transforming growth factor-β1 (TGF-β1) (n = 5 to 8 at each time point for each treatment). For quantitative measurement of MCP-1 messenger ribonucleic acid (mRNA), n = 5 to 6 at each time point for each treatment. For analysis of mortality risk and to determine risk of graft failure with E5 treatment vs PL treatment, animals that were operated on to place grafts for histologic/immunohistochemistry and mRNA analyses were grouped together with those operated on in pilot studies: n = 74 for surgeries using animals treated with estrogen and n = 57 for surgeries using animals treated with placebo.

Enzyme-linked immunosorbent assay (ELISA) for estradiol. Blood was taken from rats' tail veins at the time of implantation of the pellets, at surgery, once each week after surgery, and at harvest for the analysis of the blood serum E_2 level. The samples were centrifuged, and the serum was stored at −20° C until analysis. Serum E_2 level was measured with use of an ELISA kit (YJ Bioproducts, Rancho Cordova, Calif) per manufacturer's instructions, except that 96 half-well plates were used and reagents were diluted 1:1 in PBSGHT, which consists of gelatin (1 g/L), hemin (5 mg/L), and 0.05% Tween in 1× phosphate buffered saline (PBS). TMB (Kirkegaard & Perry Laboratories) was used for color development. Plates were read at 450 nm.

Histologic examination. Vein specimens and other tissues were fixed in 10% formalin overnight at 4° C and embedded in paraffin as described previously.[14] The proximal regions of vein grafts (0 to 3 mm from proximal anastomotic site) historically displays the most IH. This region was sectioned (5 μm) at intervals as described previously; the results reported for each graft are an average of measurements from these sections.[6]

Immunohistochemistry. Histochemistry and immunohistochemistry were performed according to previously published methods with modifications.[14] Primary antibodies used were against TGF-β1 (27235.1; R&D Systems, Minneapolis, Minn), monocytes and macrophages (ED1; Serotec, Raleigh, NC), MCP-1 (Peprotec, Rocky Hill, NJ), and PCNA (PC10; Sigma, St Louis, Mo). A control primary antibody was used to assure that the staining observed was not due to nonspecific interactions. Detection of the primary antibody and color development was performed by using the Universal DAKO LSAB 2 Kit, Peroxidase for use on RAT Specimens (DAKO, Carpinteria, Calif) and DAKO Liquid DAB Large Volume Substrate-Chromogen System according to instructions.

Reverse transcription–quantitative polymerase chain reaction (PCR). The graft was homogenized for approximately 15 seconds in 0.4 mL of ice cold Trizol (Invitrogen, Carlsbad, Calif). The mRNA was then extracted from the Trizol and reverse-transcribed to complementary deoxyribonucleic acid (cDNA).[6,15] Yields of cDNA were quantified by use of fluorescence spectrometry, and samples were diluted to 20 ng/μL. For quantitative PCR, we pipetted two standard curves (MCP-1 and the housekeeping gene hypoxanthine phosphoribosyltransferase, HPRT). Four reactions of each sample (using 1, 1, 2, and 2 μL of sample) were run for each primer set (MCP-1 and HPRT) in the same run on the Rotor-Gene 2000 (Corbett Research, Australia) as the standard curves. Cycle threshold was used to determine copies of MCP-1 per copies of HPRT. Comparing the results from reactions using 1 μL of sample to the reactions that used 2 μL of sample confirmed that the measurements were quantitative. The four estimates of MCP-1 copies per copy of HPRT were then averaged to give the result for each sample. Primers were designed to include a spice site in the amplicons as a control for the presence of genomic DNA.

Computerized image analysis and statistics. Images of vein graft cross-sections were captured with use of a Nikon microscope and Pixera color camera (Kodak, Rochester, NY). Various morphometric parameters such as total and neointimal areas and areas of positive immunostaining

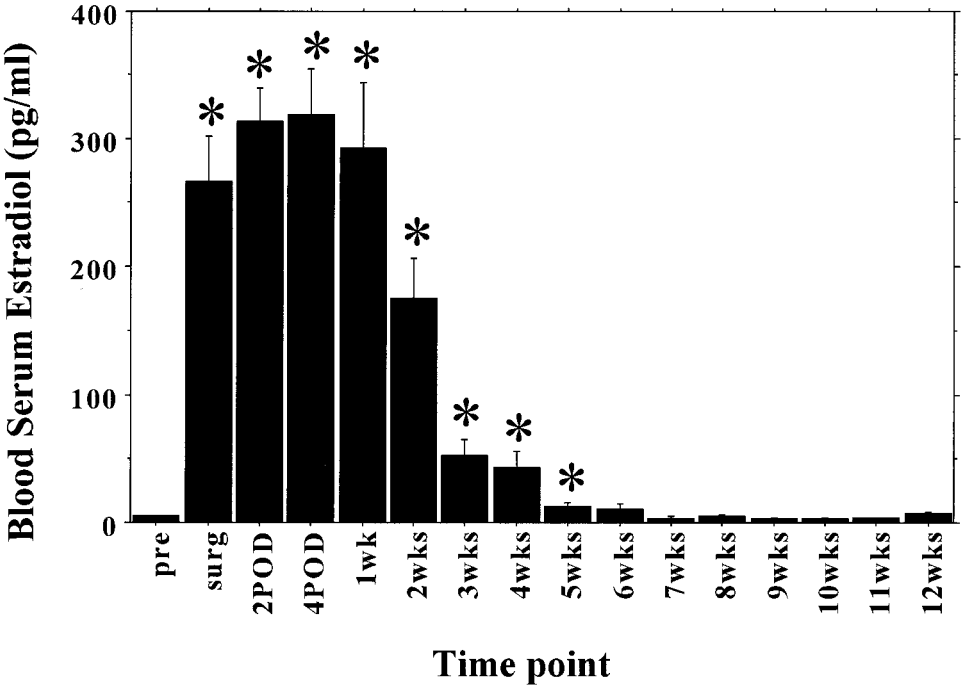

Fig 1. Serum estradiol levels increased significantly in the E5 group. On the day of surgery, 2 days after implantation of a time-release E_2 pellet, E_2 levels reached over 250 pg/mL. Although this high E_2 level was maintained for 1 week after surgery, it gradually decreased starting at 2 weeks after surgery and eventually returned to the pre-treatment level at 6 weeks after surgery. *$P < .05$, E5 group vs PL group. *Pre*, 2 days prior to surgery and the day of pellet implantation; *surg*, day of surgery; *POD*, postoperative day.

were measured by using image analysis MetaMorph software (Universal Imaging, Downington, Pa).[6,14,15] Neointimal area ratio (neointimal area/total area), immunohistochemical positively staining area ratio (area stained/total area), and message quantification were analyzed by analysis of variance (ANOVA) with Fisher protected least significant differences that compared changes over time within a treatment group, differences between groups, and interactions between time and groups. Specific time points were compared with pair-wise comparisons within the ANOVA. Data were transformed using an arcsine square-root transformation (neointimal area ratio and immunohistochemical positively staining area ratio) or log transformation (message quantification) to better meet the assumptions of ANOVA. In a second analysis, the Spearman correlation coefficients were calculated for the immunohistochemical positively staining area ratio vs the E_2 serum level at harvest and for the MCP-1 message quantification vs the E_2 serum level at harvest.

RESULTS

Animals and grafting

Overall vein graft patency rate was 90%. When we applied "proportional hazards model for grouped data,"[16] we did not observe a significant difference between the E5 and PL animals for these combined outcomes: graft patency, occlusion, or thrombosis; $P = .1238$. However, the risk ratio for graft patency, occlusion, or thrombosis in the E5 group compared to the PL group was 5.3, indicating a higher risk for graft failure in the animals receiving estradiol. Kaplan-Meier survival curves (death) followed by log-rank test yielded $P = .5248$, indicating no statistically significant difference in rat survival between the two groups. Significant body-weight loss was observed in the E5 group, a maximal difference of 11.5% compared to the PL group occurred at 1 week after surgery. This trend continued in the latter weeks. Although animals in both groups gained weight, those in the E5 group were not able to reach their presurgical weight, whereas those in the PL group did eventually exceed their presurgical average weight.

Serum 17β-estradiol level

There was no difference in serum E_2 levels between the two groups before implantation of the E_2 time-release pellet. Once E_2 pellets were implanted, E_2 levels increased to greater than 250 pg/mL on the day of surgery, which was 2 days after pellet implantation (Fig 1). E_2 levels started decreasing after 2 days, dropping down to 175 ± 31.4 pg/mL at 2 weeks and 43.0 ± 13.1 pg/mL at 4 weeks. E_2 levels returned to pretreatment levels by 6 weeks. No elevation of E_2 levels above baseline was observed in the PL group (data not shown).

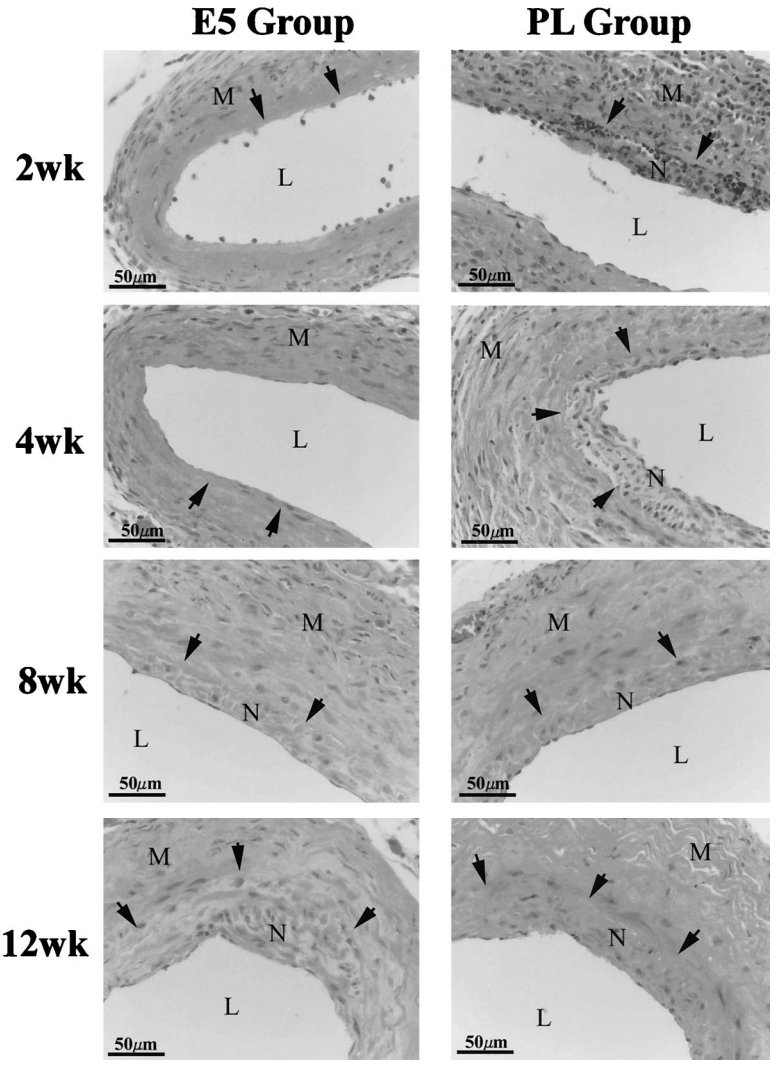

Fig 2. Neointimal formation and cell infiltration to the graft was reduced in the E5 group. More neointimal formation consisting of unorganized cells was observed in the PL group than in the E5 group at 2 and 4 weeks after surgery. Further, severe cell infiltration to the graft was seen only in the PL group at 2 weeks. At 8 and 12 weeks, which was after serum E_2 levels returned to normal in the E5 group, no differences could be seen between the two groups. *L*, Lumen; *M*, media/adventitia; *N*, neointima. *Arrow heads* indicate the internal elastic lamina. Magnification, ×400.

Intimal hyperplasia was reduced with 17β-estradiol treatment

Cross-sections stained with hematoxylin and eosin revealed that an eccentric neointimal formation consisting of unorganized cells was produced inside the internal elastic lamina (IEL). The greatest inflammatory cell infiltration was observed in 2 week-grafts in both the PL and E5 groups (Fig 2). Neointimal area ratio (NR) was calculated as the neointimal area over total graft area, and is presented in Fig 3. NR was increased with graft age in both groups and was reduced significantly at 2 and 4 weeks after surgery in the E5 group compared to the PL group (2 weeks—E5 = 6.9 ± 1.7%, PL = 12.6 ± 1.5%; $P < .05$. 4 weeks—E5 = 8.5 ± 2.0%, PL = 26.7 ± 7.4%; $P < .05$). There were, however, no significant differences at 8 and 12 weeks, which is when serum E_2 returned to pre-treatment levels.

Immunohistochemistry

ED1 (monocytes/macrophages). At 2 weeks, transmural macrophage infiltrations in the grafts were observed as well as accumulation along the IEL. In the E5 group, less macrophage accumulation in the IEL was observed compared to PL group, which had many macrophages along the IEL as well as in the media/adventitia (Fig 4). A quantitative analysis was performed of the ED1 area ratio, which was calculated by ED1 positive area over total area (Fig 5, *A*). ED1 area reached a maximum at 4 weeks after surgery in the PL group. It is important to note that in the E5

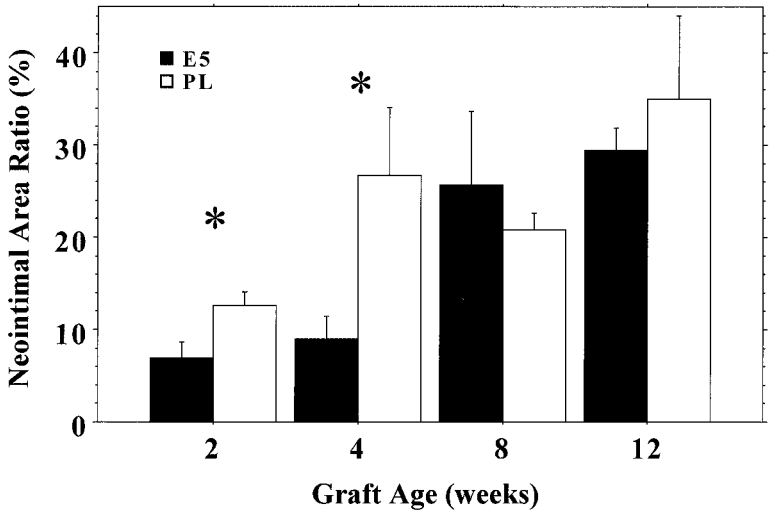

Fig 3. Neointimal area was significantly reduced in the E5 group. Neointimal area ratio was calculated as neointimal area over total area. At 2 and 4 weeks after surgery, neointimal area ratio was significantly reduced in the E5 group vs the PL group. On the other hand, there were no significant differences between the two groups at 8 and 12 weeks when serum E_2 returned to pretreatment levels. *$P < .05$, E5 group vs the PL group.

group, ED1 staining was significantly reduced compared to the PL group at 2 and 4 weeks by 73.1% and 88.3%, respectively. ED1 staining peaked in the E5 group at 8 weeks, after serum estradiol levels had returned to baseline (Fig 5, *A*). There was no significant difference between the two groups at 8 and 12 weeks. When time was removed as a factor, the ED1 area ratio correlated inversely with the blood serum E_2 level at graft harvest ($r = -0.46$, $P = .0007$).

Proliferating cell nuclear antigen. In the E5 group, many fewer PCNA-positive cells were observed compared to the PL group, which had many positive cells in the media and adventitia (Fig 4). The ratio of proliferating cell-positive area was significantly reduced in the E5 group vs the PL group at 2 and 4 weeks after surgery. PCNA staining peaked in the E5 group at 8 weeks, after serum estradiol levels had returned to baseline (Fig 5, *B*). There was no significant difference between groups at 8 and 12 weeks. When time was removed as a factor, the PCNA area ratio was correlated inversely with the blood serum E_2 level at graft harvest ($r = -0.59$, $P = .0001$).

Monocyte chemoattractant protein 1. Less MCP-1 positive stain was detected in the E5 group than in the PL group (Fig 4). The MCP-1–positive cells in the PL group were observed either in media or in neointima, so that these cells were likely smooth muscle cells/myofibroblasts, endothelium cells, and macrophages, which are known to be major sources of MCP-1. In this quantitative analysis, MCP-1 expression reached a maximum at 2 weeks after surgery and then gradually decreased in the PL group. MCP-1 expression was reduced significantly at 2 and 4 weeks in the E5 group compared to the PL group, by 95.6% and 75.7%, respectively (Fig 5, *C*). When time was removed as a factor, the MCP-1 area ratio was inversely correlated to the serum E_2 level at graft harvest ($r = -0.43$, $P = .0026$).

Transforming growth factor-β1. TGF-β1–positive stain was seen in the endothelial cells and media and adventitia in both the E5 and PL groups (Fig 4). Quantitative analysis showed no significant difference between the two groups at any time points (Fig 5, *D*). TGF-β1 was highest at the earliest time point examined (2 weeks) and then showed a trend toward reduced expression with time. When time was removed as a factor and the TGF-β1 area ratio was compared to the serum E_2 level at graft harvest, $r = -0.22$ and $P = .14$ (ie, the relationship did not reach statistical significance).

Quantitative polymerase chain reaction

Message analysis with quantitative PCR showed that MCP-1 message expression was attenuated at 2 and 4 weeks after surgery in the E5 group compared to the PL group by 43.0% and 33.1%, respectively. Although ANOVA indicated that the attenuation was significant at 4 weeks ($P = .015$), the attenuation at 2 weeks was not statistically significant ($P = .48$). No attenuation of MCP-1 message expression was observed at 8 weeks after surgery in the E5 group compared to the PL group after serum E_2 levels returned to baseline in the E5 group (Fig 6). When time was removed as a factor and the MCP-1 message expression was compared to the serum E_2 level at graft harvest, $r = -0.18$ and $P = .32$ (ie, the relationship did not reach statistical significance). The lack of correlation is consistent with ANOVA results at 2 weeks (ie, even though serum E_2 levels were high at the 2-week time point there was not a statistically significant reduction in MCP-1 message expression).

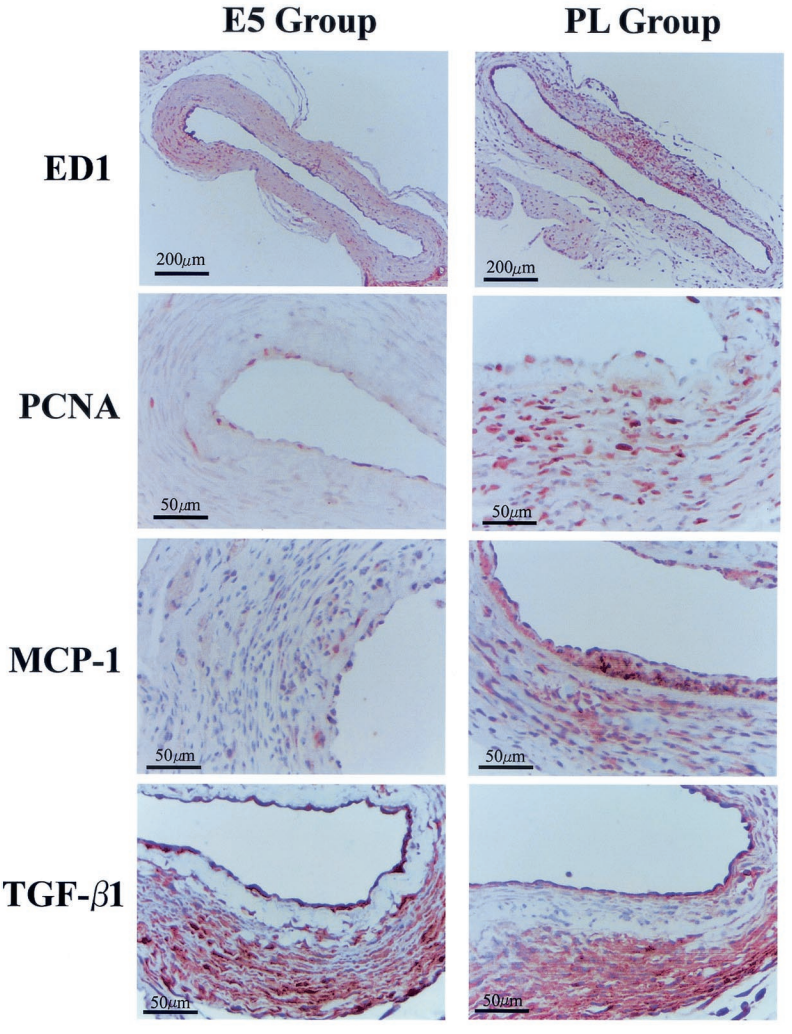

Fig 4. Accumulation of macrophage/monocytes and proliferating cells, and expression of MCP-1 were attenuated at 2 weeks after surgery in the E5 group. Macrophages, indicated as ED1 positive cells, accumulated to the internal elastic lamina in the E5 group. Much macrophage accumulation was shown along the internal elastic lamina as well as in the media in the PL group. Many proliferating cells, indicated as PCNA positive cells, were observed in media in the PL group compared to the E5 group. MCP-1 was much higher in the neointima and media in the PL group compared to the E5 group. Endothelium and media had much TGF-β1 positive stain in both groups. All photomicrographs at ×400 magnification except ED1 stain, which is shown at ×100.

DISCUSSION

Leukocytes, macrophages, and their cytokine products are involved in the development of neointimal hyperplasia. We have reported that rats treated with *L-Cl2MBP*, which depletes monocytes and macrophages, demonstrate significantly reduced IH at 7 and 14 days after vein grafting.[17] Saito and colleagues[10] have stated that both macrophage accumulation and IH were significantly reduced in rat aortic allografts among animals receiving E_2 treatment. Thus, we hypothesized that E_2 treatment might inhibit vein graft IH coincident with a reduction of MCP-1 expression and accumulation of macrophages within the graft.

We targeted an E_2 level between 150 and 200 pg/mL because E_2 levels of 152 ± 24.9 pg/mL[10] or near 200 pg/mL[13] have been reported to reduce IH in rat aortic allografts and in rat arteries following balloon injury, respectively. We found that the subcutaneous pellets resulted in an E_2 level near 250 pg/mL at the day of surgery. The time-release pellets, however, kept the E_2 level in our planned therapeutic range for only 3 weeks. IH was reduced significantly at 2 and 4 weeks, under the influence of therapeutic or near-therapeutic levels of E_2. At 6 weeks after surgery, blood serum E_2 levels did not differ significantly from pretreatment levels. This low level of E_2 nega-

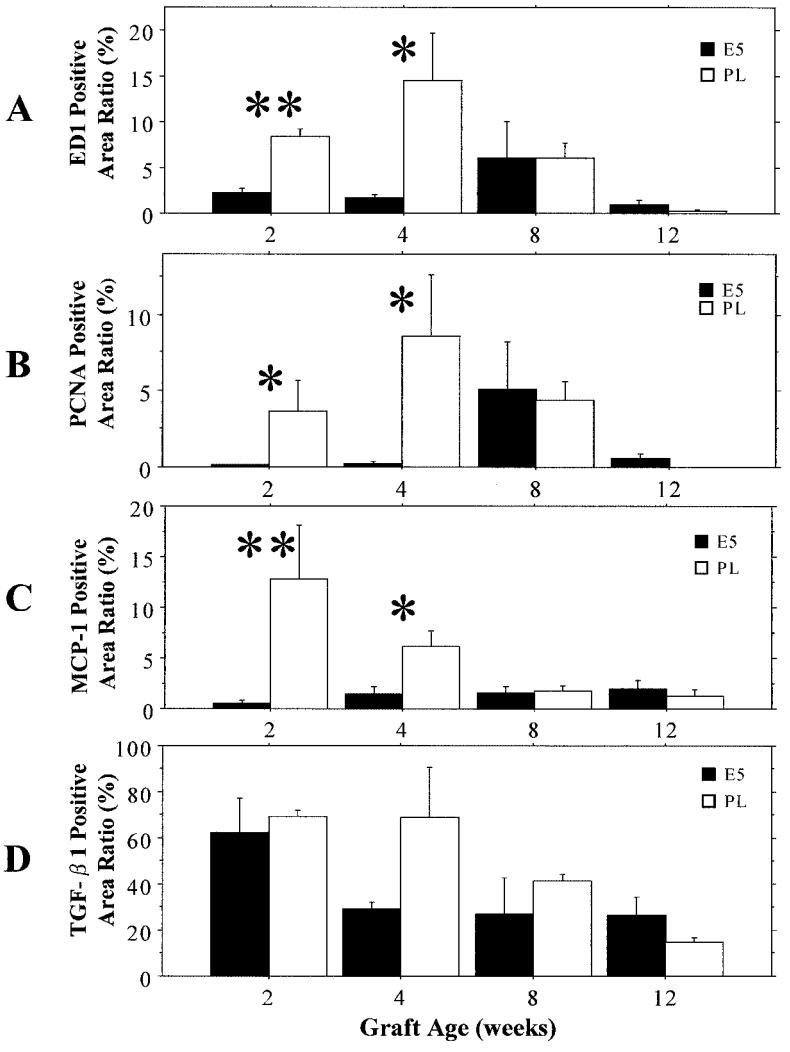

Fig 5. Number of macrophage/monocytes and proliferating cells accumulating to the graft, and expression of MCP-1 were significantly attenuated at 2 and 4 weeks after surgery in the E5 group. Quantitative analysis of images of immunohistochemically stained vein graft cross-sections is presented. **A**, ED1 positive area ratio was significantly reduced in the E5 group compared to the PL group at 2 and 4 weeks. ED1 accumulation reached a maximum at 4 weeks in the PL group. **B**, PCNA stain revealed a significant difference of positive area at 2 and 4 weeks between the two groups. PCNA positive cell area ratio also reached a maximum at 4 weeks in the PL group. **C**, MCP-1 expression was significantly inhibited in the E5 group at 2 and 4 weeks after surgery. In the PL group, the peak MCP-1 expression was observed at 2 weeks and later decreased gradually. **D**, TGF-β1 stain did not reveal significant differences at any time points. $*P < .05$, $**P < .01$; E5 group vs PL group.

tively influenced our results at the 8- and 12-week timepoints when intimal areas were similar between the E5 and PL groups. It is of interest that the difference in neointimal ratio established at 2 and 4 weeks was lost at 8 and 12 weeks, implying that a short treatment with E_2 at the time of vein grafting would not be clinically effective.

E_2 treatment resulting in inhibition of postinjury neointimal hyperplasia in arterial models has been well described.[10-13,18] To our knowledge, prior to our present work, only one report exists in the literature that examines the effects of E_2 in an in vivo vein graft model of IH. Calcagno and colleagues[19] reported that E_2 treatment (serum level, 600 pg/mL) was not effective in inhibiting intimal hyperplasia in a rabbit vein graft model. They reported that one graft thrombosed immediately as the result of a technical problem, whereas 7 of 12 grafts were found to occlude when harvested at 3 weeks in the E_2-treated group. E_2 did not significantly reduce IH in their limited study. In our study, the neointimal ratio was reduced significantly in the E5 group compared to the PL group: by 24.6% at 2 weeks and 33% at 4 weeks, with a slight increased risk of occlusion. Gerstman and col-

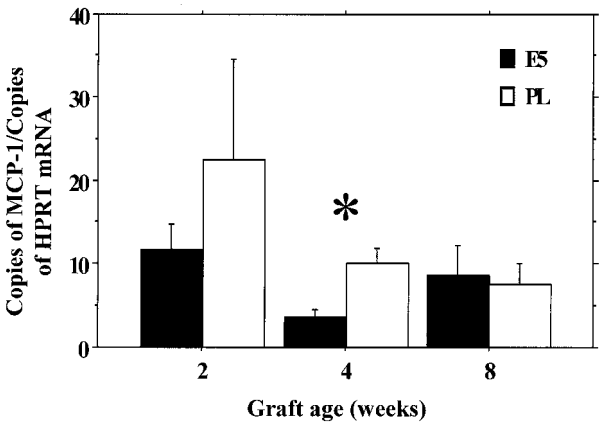

Fig 6. In the E5 group, MCP-1 mRNA expression showed a trend toward attenuation at 2 weeks and was significantly inhibited at 4 weeks compared to the PL group. However, there was no difference between the groups at 8 weeks, after the serum E_2 level returned to baseline. Results are from reverse transcription of mRNA followed by quantitative PCR based on cycle threshold vs standard curves of known copies of the genes. Data shown normalized to the housekeeping.

leagues[20] reported an increased risk of deep vein thrombosis related directly to the dose of estrogen in humans. Perhaps the high incidence of thrombus generation at an E_2 serum level of 600 pg/mL reported by Calcagno and colleagues overwhelmed any beneficial effects of estrogen treatment on IH in patent grafts and contributed to the observed graft failures.

MCP-1, which is one of the pivotal chemokines involved in the inflammatory system, is known to cause macrophages to migrate and accumulate. Macrophages are capable of secreting numerous growth factors, which may influence the development of IH. Once endothelium or VSMCs are injured, MCP-1 is secreted by these cells.[6] VSMCs in our epigastric vein femoral artery interposition graft model undergo necrosis and apoptosis between 1 and 10 days after surgery.[21,22] Alpha-actin staining cells with the ultrastructural appearance of myofibroblasts appear in the adventitia by 4 to 10 days and repopulate the neointima by 4 weeks.[21] Our immunohistochemical analysis revealed that MCP-1 expression was reduced significantly in the E5 group at 2 and 4 weeks after surgery, when serum E_2 levels were elevated. At 4 weeks, the greatest reduction in MCP-1 staining occurred in the neointima and media/adventitia adjacent to the IEL in regions populated with myofibroblasts. Reduction in MCP-1 correlated with decreased macrophage accumulation. At 2 weeks, the reduction in MCP-1 staining seen transmurally in the E5 group is likely secondary to decreased macrophage infiltration and proliferation. Furthermore, our MCP-1 message analysis showed MCP-1 mRNA expression had a tendency of attenuation at 2 weeks and was reduced significantly at 4 weeks in the E5 group compared to the PL group, although there was no difference between the two groups at 8 weeks after serum E_2 levels returned to baseline. These results were consistent with our immunohistochemistry results. Finally, when a comparison was made between serum E_2 levels at harvest of the graft and the percentage of cells in that graft staining positive for ED1, PCNA, or MCP-1, a negative Spearman correlation coefficient was obtained, indicating that elevated levels of serum E_2 were correlated with lower levels of these indicators of IH.

How E_2 affects MCP-1 expression is unclear and the focus of future experiments. Estrogen receptor expression has been demonstrated by VSMCs and endothelial cells.[23,24] Yue and colleagues[25] reported that the estrogen receptor modulator, idoxifene, inhibited neointimal formation in a rat balloon arterial injury model by decreasing VSMC proliferation. Kauffman et al[26] demonstrated that raloxifene, a second estrogen receptor modulator, also decreased intimal thickening following arterial injury. Kauffman et al reported that raloxifene's beneficial effect was blocked when treating their rats with a pure estrogen receptor antagonist, ICI 182,780. However, pretreatment with the receptor antagonist did not completely block the reduction in intimal areas seen with E_2 treatment. Thus it is likely that estradiol's effect on the vascular wall is in part receptor–mediated but that other mechanisms independent of estrogen receptors may be involved.

CONCLUSION

17β-Estradiol inhibited vein graft IH coincident with a reduction in the expression of MCP-1. Estradiol treatment reduced cellular proliferation and decreased macrophage infiltration of rat vein grafts. Future studies will be directed toward determining the cell types and cell biology influenced by estradiol. Our goal will be to gain sufficient knowledge to determine whether the attenuation of IH by estradiol or other estrogen receptor agonists can be made permanent within an acceptable clinical safety profile.

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